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Effect of vegetable oils on the methane concentration and population density of the rumen ciliate, *Eremoplastron dilobum,* grown *in vitro*

A. Cieślak[1,3], R. Miltko[2], G. Bełżecki[2], M. Szumacher-Strabel[1], A. Potkański[1], E. Kwiatkowska[2] and T. Michałowski[2]

[1]The August Cieszkowski Agricultural University of Poznań,
Department of Animal Nutrition and Feed Management
Wołyńska 33, 60-637 Poznań, Poland
[2]The Kielanowski Institute of Animal Physiology and Nutrition,
Polish Academy of Sciences
05-110 Jabłonna, Poland

ABSTRACT

The effect of rapeseed (RSO) and linseed (LSO) oils on the methane concentration and number of rumen ciliates (*Eremoplastron dilobum)* incubated *in vitro* was investigated. A 5% dose of rapeseed oil reduced the methane concentration by about 14% ($P<0.05$) but had no effect on the ciliate number. Conversely, the same dose of LSO significantly increased the protozoa count, while methane production remained unchanged. No positive correlation was found between the methane concentration and ciliate number or with the inhibitory effect of oils rich in C18:1, C18:3 fatty acids on the population density of *Eremoplastron dilobum*.

KEY WORDS: *Eremoplastron dilobum*, vegetable oils, methane emission, fermentation *in vitro*

INTRODUCTION

Methanogens associated with ciliate protozoa are responsible for 9-25% of the total methane production in the rumen, thus they significantly contribute to the methane emission from ruminant production (Hergarty, 1999). Eleven species of entodiniomorphid protozoa, including some species from the genus *Eremoplastron,* have been found to have adhered methanogens (Karma, 2005). Vegetable oils are considered to be a natural source of unsaturated fatty acids (UFA) influencing the

[3] Supported by National Scholarship the Foundation for Polish Science
[3] Corresponding author: e-mail: adamck@jay.au.poznan.pl

protozoa count and, consequently, ruminal methanogenesis. There is conflicting evidence suggesting both a stimulatory and a toxic effect of long-chain fatty acids on ruminal protozoa (Ivan et al., 2001; Kišidayová et al., 2005). The objectives of the present study were to investigate the effect of rapeseed and linseed oils rich in C18:1 and C18:3 fatty acids on methane production and the number of the rumen ciliate, *Eremoplastron dilobum,* during 24 h incubation *in vitro.*

MATERIAL AND METHODS

The ciliates, *Eremoplastron dilobum* (Kofoid and Mc Lennan, 1932), were isolated from the rumen fluid of sheep and cultured *in vitro* according to Michałowski et al. (1986) in a chemically defined medium consisting of, g/l: K_2HPO_4 3.48, $NaHCO_3$ 2.1, NaCl 0.76, $CaCl_2 \cdot 6H_2O$ 0.33, CH_3COONa 6.12, $MgCl_2 \cdot 6H_2O$ 0.30, $NaH_2PO_4 \cdot 6H_2O$ 1.59 and Na_2HPO_4 1.71 (Michałowski et al., 1999). Every day a mixed feed was added to the protozoa culture (0.375 mg/ml culture/d). The feed was composed of powdered, %: meadow hay 60, wheat gluten 16, crystalline cellulose 12, and barley flour 12. The fermentation study was performed using serum bottles. The basal medium composed of 20 ml of culture medium and 16 mg of substrate (Table 1) given alone (control samples) or supplemented with either RSO or LSO (experimental samples) was inoculated with 20 ml of ciliate suspension and incubated for 24 h at 39°C. The following parameters were measured in the post culture medium: pH, volatile fatty acids (VFA) and methane levels. VFA and methane were quantified by gas chromatography, whereas ammonia, spectrophotometrically using the Nessler reagent. Ciliates were counted as described by Michałowski et al. (1986). The composition of the basic feed mixture per control incubate was, mg: hay 9.0, wheat gluten 2.4, cellulose 1.8, and barley flour 1.8, and was supplemented with 0.66 mg of rapeseed or linseed oil in the respective experimental incubates.

The obtained data were subjected analysis of variance using the general linear model (GLM) according to the procedures of the SAS program (version 9.1, SAS Institute Inc., Cary NC).

RESULTS AND DISCUSSION

Neither rapeseed nor linseed oil influenced the pH values or ammonia concentration in the culture medium (Table 1). The concentration of VFA ranged between 709 and 1288 μM/100 ml, but the molar proportions of the particular acids remained unchanged and exhibited a bacterial rather than protozoan

fermentation pattern (Michałowski, 1987). This suggests that the majority of VFA was of bacterial origin. Rapeseed oil reduced methane production but did not affect the ciliate count. Conversely, linseed oil increased the population density of *Eremoplastron dilobum,* but had no effect on the methane concentration.

Table 1. Effect of vegetable oil on the pH of the post culture medium, ammonia, volatile fatty acids and *Eremoplastron dilobum* during of 24 h *in vitro* culture. Mean values (n= 6)

Item	Treatment			
	control	RSO	LSO	SEM
	N			
pH	6.73[a]	6.70[a]	6.70[a]	0.01
Ammonia, mmol/l	1.49[a]	1.65[a]	1.75[a]	0.08
Total VFA µM/100 ml	816.80[a]	709.15[a]	1288.92[a]	180.23
Acetate, %	72.61[a]	65.16[a]	79.59[a]	6.53
Propionate, %	18.61[a]	22.84[a]	13.30[a]	4.38
Butyrate, %	8.74[a]	11.90[a]	7.11[a]	2.19
Methane, mM^{-1}	1.03[a]	0.89[b]	1.01[a]	0.04
Ciliate number, ml^{-1}	1252[b]	1270[b]	1480[a]	40.30

values with the same letter are not significantly different ($P<0.05$)

It has been shown that the effects of vegetable-oil-derived UFA on ruminal ciliates may vary depending on the protozoa species. It is difficult to compare the results of studies dealing with different species as well as different fatty acids. The most pronounced toxic effect of vegetable oil was observed in relation to holotrich ciliates. The population of *Eremoplastron bilobum* was, however, significantly reduced in the presence of microbial oil (MO), evening primrose oil (EPO) and borage oil (BO). All of them are rich in C18:2 or C18:3 fatty acids. In contrast to this, MO and EPO increased the population density of *Entodinium* spp. cultured *in vitro* (Kišidayová et al., 2006). A decrease in the protozoa count resulting from a high fat content in the diet has been reported in several studies (Ivan et al., 2001; Hristov et al., 2004). In the present study, a positive effect of vegetable oil rich in C18:1 and C18:3 on the number of *Eremoplastron dilobum* was found only in the case of LSO. No negative influence of RSO was, however, observed. A similar influence of rapeseed oil on the total protozoa count in the rumen was found by Tesfa (1993). In contrast, Hristov et al. (2004) observed a strong antiprotozoal effect of C18:3 derived from linseed oil on a wide range of protozoa species. The results of the present study showed no correlation between the methane concentration and ciliate number. This may suggest that other microbial populations like methanogenic archaea were also modified, as previously pointed out by Firkins et al. (2006). It would be interesting to compare the response of other species of rumen entodiniomorhids to feed supplemented with vegetable oils rich in C18:1 and C18:3 fatty acids.

CONCLUSIONS

This study showed that vegetable oils rich in C18:1, C18:3 fatty acids differently affected the number of the rumen ciliate, *Eremoplastron dilobum,* grown *in vitro*. No relationship was also found between the number of ciliates and methane production. This suggests that other, undefined, factors influencing the activity or/and the number of methanogens should also be considered. Further studies thus seems necessary to explain the role of UFA in regulation of rumen protozoa growth and their involvement in methane production.

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